Trichomonas vaginalis Quantitative qPCR Standard

Catalogue number	Presentation
MD07211	0,1 mL

Description

The Trichomonas vaginalis Quantitative qPCR Standard is a highly specific product designed for quantitative real-time PCR (qPCR) applications, and it is explicitly tailored for use in conjunction with the Trichomonas vaginalis qPCR Kit (MD05911). The essence of this product resides in its provision of a fragment from *Trichomonas vaginalis*, meticulously measured to be at 2 x 10^6 copies/µL. When a quantitative analysis of *T. vaginalis* samples is required, a standard curve should be generated using T. vaginalis Quantitative qPCR Standard and analysed in a qPCR run. Serial dilutions of the Quantitative Standard must be meticulously prepared with a defined genome copies per microliter (copies/µL). The data obtained from these serial dilutions is then plotted to form a standard curve, which serves as a reference for determining the concentration of *T. vaginalis* in the test sample. qPCR method serves as a gold standard in molecular diagnostics due to its exceptional accuracy, specificity, and sensitivity. Please be reminded that this kit is designed for use by trained laboratory personnel and should be used strictly in accordance with the accompanying Instructions for Use (IFU) to ensure accurate and reliable results. Execution of the outlined procedures is crucial in minimizing potential errors and maintaining the integrity of the testing process.

Shipping & Storage Conditions

This product can be shipped at a range of temperatures from dry ice to room temperature (RT). Although kit components are stable at room temperature, they should be immediately stored at -85°C to -15°C upon arrival. Also proceed with the following recommendations:

- Minimise the number of freeze-thaw cycles by storing kit components in working aliquots.
- If the package that protects the kit arrived damaged, please contact NZYtech.
- Beware of the expiry date indicated on the packaging. NZYtech does not recommend using the kit after the expiry date. On this date, the Quantitative qPCR Standard must be discarded.

Components

The kit provides a comprehensive set of reagents, allowing for the generation of multiple quantitative Standard Curves of significance:

Component	Tubes	Volume	Cap color
T. vaginalis Quantitative Standard (2 x 10^6 copies/µL)	1	100 µL	Red
Positive Control Dilution Buffer	2	1500 µL	Green

Reagents, Materials and Equipment Required but Not Provided

- This Quantitative qPCR Standard is to be used solely with NZYtech's Trichomonas vaginalis qPCR Kit (MD05911).
- Commercially available Real-time PCR Instruments that detect FAM™ and HEX™/JOE™/VIC™ fluorescent dyes (emission wavelengths of 520 and 556/555/554 nm, respectively).
- RNase/DNase-free qPCR plasticware: PCR tubes, strips, caps, 96-well plates, and adhesive films (also available at NZYtech).
- Pipettes and filter tips (RNase/DNase-free).
- Disposable gloves.
- Vortex and centrifuge.

Standard Protocol

Nucleic Acids Extraction

Initiate sample preparation as delineated in the IFU associated with the Trichomonas vaginalis qPCR Kit (MD05911). Please note that for quantitative applications, it is advised against the pooling of samples, as this could introduce variability and affect the accuracy of quantification. All dilutions conducted with the sample need careful consideration, particularly if the end goal is to extrapolate the initial copy number quantification based on the starting volume or mass of the sample. This diligence ensures a reliable and accurate representation of *T. vaginalis* concentration in the original specimen, crucial for the validity of the subsequent qPCR analyses.

Procedure

Follow the IFU associated with the Trichomonas vaginalis qPCR Kit (MD05911) while ensuring to incorporate the reactions for additional seven serial dilutions of the T. vaginalis Quantitative Standard into the assay. To prepare the nucleic acids for the Quantitative Standard curve, proceed as follows:

1.1 Pipette 90 µL of Positive Control Dilution Buffer into 6 tubes labelled 2 to 7.
1.2 Pipette 10 µL of T. vaginalis Quantitative Standard (2 x 10^6 copies/µL) into tube 2.
1.3 Vortex thoroughly and spin.
1.4 Change tip and pipette 10 µL from tube 2 into tube 3.
1.5 Vortex thoroughly and spin.
1.6 Repeat steps 1.2 (from sequential tubes) to 1.5. to complete the dilution series. The final Standard Curves have the following copy number per reaction:

Standard Curve	Copy number (per µL)	Copy number per reaction (per 5 µL)
Tube 1 - T. vaginalis Quantitative Standard	2 x 10^6	10^7
Tube 2	2 x 10^5	10^6
Tube 3	2 x 10^4	10^5
Tube 4	2 x 10^3	10^4
Tube 5	2 x 10^2	10^3
Tube 6	20	10^2
Tube 7	2	10

1.7 Pipette 5 µL of each dilution into each well containing 15 µL of reaction mix, according to your experimental plate set-up (prepare reaction mix according with Trichomonas vaginalis qPCR Kit (MD05911) IFU). The final volume in each well should be 20 µL. To minimize the risk of potential cross-contaminations, Quantitative Standards should be added to the corresponding qPCR well plate after all previous samples and control reactions are set up.
1.8 Proceed with qPCR as recommended in the IFU of the respective kit.

Quality Control

Nucleases assay

To test for DNase contamination, 0.2-0.3 µg of pNZY28 plasmid DNA are incubated with the kit component in test for 14-16 h at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with the kit component in test for 1 h at 37 °C. Following incubation, the nucleic acids are visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acids.

Functional assay

The qPCR/RT-qPCR reactions must ensure the consistent amplification of target DNA/RNA across serial dilutions, meeting specified acceptance criteria for assay performance.

Data analysis

Prior to proceeding with the analysis of sample results, we strongly advise validating the integrity of the real-time PCR test. This can be achieved by ensuring that both Positive and Negative controls, along with the Internal Extraction Controls (IEC), have performed as anticipated, as outlined in the kit's Instructions for Use (IFU).

The processing of the amplification data can be conducted automatically, utilizing the dedicated software tool that accompanies your Real-Time PCR thermal cycler. However, should you opt for a more manual approach, the threshold should intersect with the FAM curve in its linear phase of increase (using the log scaling of the y-axis). This setting of the threshold thereby dictates the Crossing Point (Cp) or Cycle threshold (Ct), which is inversely correlated with the initial concentration of target gene copies' present in the Real-Time PCR reaction.

It is important to note that only curves demonstrating a typical exponential amplification should be considered positive. These are characterized by an initial flat baseline, followed by a clear exponential increase in fluorescence, potentially culminating in a plateau phase when viewed with a logarithmic y-axis scaling.

In the subsequent figure, you can find a typical Standard Curve that has been generated using this kit:

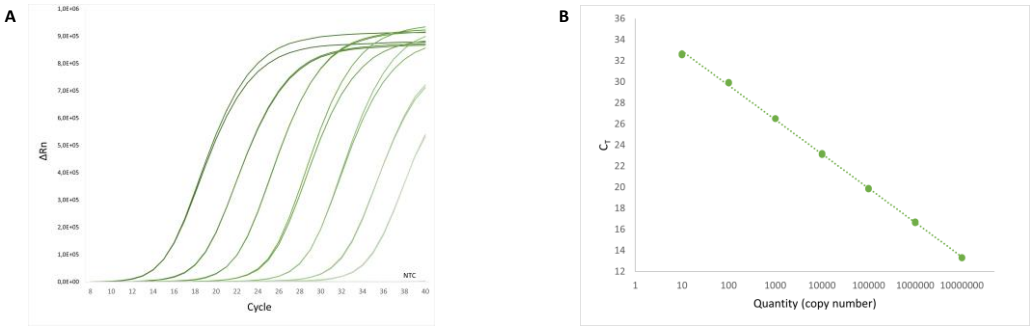

Figure 1: Amplification plot (cycle number versus fluorescence - ΔRN) **(A)** and the respective standard curve (copy number versus Ct) **(B)** of 1:10 serial dilutions of T. vaginalis Quantitative Standard, from 1 x 10^7 copies to 10 copies per reaction detected through the FAM channel using Trichomonas vaginalis qPCR Kit (MD05911). NTC, No Template Control (negative control).